

Reiko YOROI*: **Spore germination and gametophyte
development of the Vittariaceae (1)**

鑑 礼子*: シシラン科の孢子発芽と配偶体 (1)

Many fern gametophytes included in several diverse taxonomical groups have been described up to date. Some of the gametophytes have been used for various biological investigations. Informations on non-cordate prothallium, however, has been left in very poor conditions. This type of prothallia is found casually in several different families. They are, for example, Hymenophyllaceae²⁾¹⁸⁾, Pteridaceae⁸⁾, Davalliaceae¹⁾, Aspidiaceae¹⁾, Grammitidaceae¹⁵⁾, Polypodiaceae¹²⁾¹⁴⁾ and Vittariaceae²⁾⁷⁾ (all in the sense of Copeland⁴⁾⁵⁾). Despite of insufficient informations hitherto made, Copeland⁴⁾ gave a short comment on the relationship between Hymenophyllaceae and Vittariaceae based on the prothallial phase. Considering this condition, the author has been studying the gametophyte development of Vittariaceae during these four years.

Goebel⁹⁾¹⁰⁾ described gametophytes of four species in *Vittaria*, and one species from both *Monogramma* and *Hecistopteris*. Britton and Taylor³⁾ gave an account on the gametophyte of *V. lineata* J.E. Smith. Momose¹¹⁾ published detailed drawings of germinating spore of *V. zosterifolia* without mentioning the later stages. Some studies⁶⁾¹⁶⁾¹⁷⁾ in this line followed occasionally. Recently, Farrar⁷⁾ described the gametophytes of three species from *Vittaria* and one from *Ananthacorus* with special reference to gemma-formation.

Though rather scanty studies cited above outlined the prothallial features of vittarioid ferns, the process of the gametophyte development was overlooked. In this paper the growth and development of *Vittaria Fudzinoi* Makino, endemic species in Japan and China, was studied through the culture of prothallia.

Materials and methods The spores used here were collected from

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Omogokei valley, Ehime Prefecture on 22nd, October of 1970 by kind courtesy of Mr. S. Serizawa of Tokyo Kyoiku University. The cultures of the spores were set about in November.

The spores were collected from fresh fronds on smooth paper and reserved in a refrigerator after being dried in the shade for several hours. The germination rate was not affected by cold preservation within three years after collection, thus the spores being available for experiment at any time.

The spores were sown on two kinds of media: the one was solidified Meyer's solution with 0.8% agar in petri-dishes and the other was a sterilized soil in flower pots. The Meyer's solution is formulated as follows: KH_2PO_4 1.0 g, MgSO_4 0.3 g, CaCl_2 0.1 g, NaCl 0.1 g, FeCl_3 1 drop, NH_4NO_3 1.0 g, water 1 liter.

The dishes and pots were placed in a culture box kept in $23 \pm 1^\circ\text{C}$ under 12 hours illumination with fluorescent lamp at 300-1000 lux. The half the number of pots were placed in a greenhouse. Four or five months from the beginning of culture, the young gametophytes growing on agar medium were transplanted on bog-moss or soil in pots, a half number of which was placed in the greenhouse in the same manner as the preceding.

The gametophytes attained to the maturation within two years in the pots filled either with bog-moss or soil. It may reasonably be concluded that conditions were favorable for growth, since the cultures have proceeded to sexual maturity and fertilization.

Camera lucida drawings and photographs were used for studying the external morphology of the fresh materials.

Observation (1) *Vittaria Fudzinoi* Makino The gametophytes were kept alive for more than two years. They finally grew into a ribbon-like thallus of about 10 mm in length, with the apical or marginal widened portions subdivided into several short lobes, producing gemmae and sex organs on their margins. Young sporophytes were also developed from the prothallia.

1) Spore germination and filamentous stage: The yellow monolet spores have a smooth and thin exine and are devoid of perine. The average diameter of the spores is 67μ and their laesurae are long (Fig. A. 1).

In culture, the spore germinates within one month after sowing. After several days from the sowing, yellow oil-globules appear in the spore.

Finally one large globule is formed near the nucleus in the spore. When the exine partially splits at the laesura as the spore swells, a basal cell cuts off a rhizoidal cell by the first division which occurs near the laesural region parallel to the equatorial diameter of spore mother cell (Fig. A. 2, 3). The prothallial cell is produced from the basal cell by the second division at right angles to the first wall (Fig. A. 4). As the prothallial cell develops, pale green chloroplasts appear in both the prothallial and basal cells.

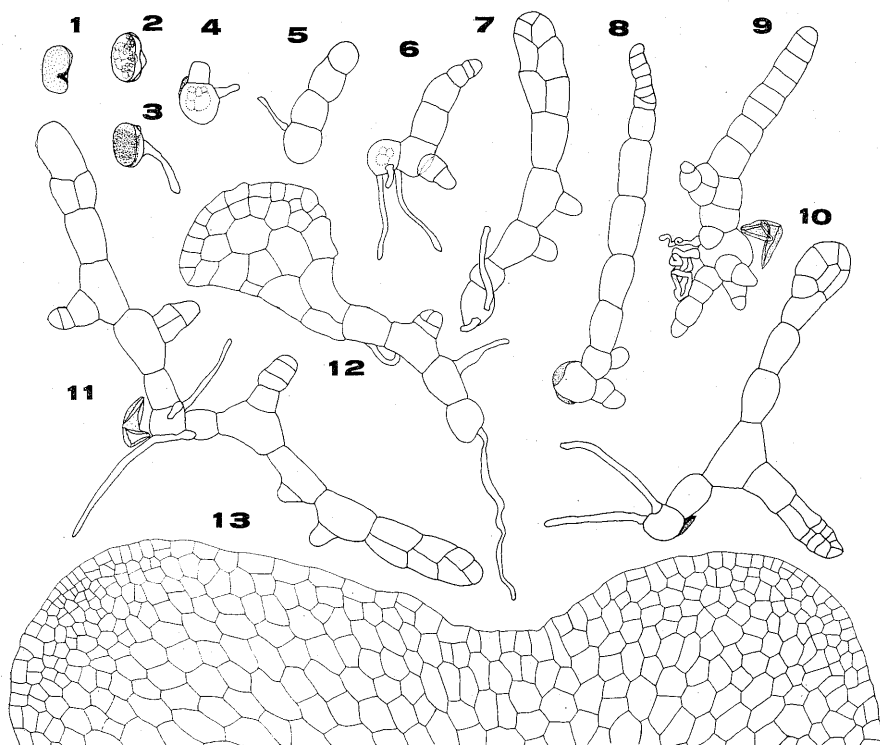


Fig. A. Spore and early stage of gametophyte ($\times 75$). 1. Spore. 2-3. 1 month after sowing, germination with rhizoidal cell. 2. Early stage. 3. Later stage. 4-5. 2 months. 4. First prothallial cell protruding. 5. Prothallial filament. 6-12. 3 months. 6. Branching of filament. 7. Longitudinal division of filament. 8. Intercalary cell-divisions of filament. 9. Basal cell producing many filaments with coiled rhizoids. 10. Spatulate stage. 11. Branching of filament with longitudinal division. 12. Formation of marginal meristematic part. 13. 1 year, marginal meristems of thallus. (Sparsely dotted areas denote spore coat).

Several medium-sized yellow oil-globules remain for more than three months in the basal cell.

Two or three months after sowing, germ filament is composed of two to ten cells through intercalary divisions (Fig. A. 5, 6, 8, 9). The basal cell often emits two to several filaments after germ filament (Fig. A. 8, 9). Sometimes more than one rhizoidal cell occur from the basal cell (Fig. A. 6, 7). Very rare case was found in which rhizoids are coiling (Fig. A. 9). Branching occurs laterally from the cell midway of the filament (Fig. A. 6-8).

2) Thalloid stage: As shown in Figs. A and B, the uniseriate filamentous prothallium (Fig. A. 5, 6, 8, 9) finally transforms into irregularly lobed shape (Fig. B. 1-3) through biseriate filament with an apical cell (Fig. A. 7, 10, 11), one cell-layered spatulate form (Fig. A. 10), and obovate obviously round-topped small plate-like structure with a filamentous stipe (Fig. A. 12).

In the meantime, in the round-topped marginal portion of the thallus, there occurs differentiation of highly meristematic parts and non-meristematic parts. These parts are often different in their sizes and sequences of occurrence, causing irregular branching. Theoretically this kind of branching involves "polychotomous" branching. As a rule by partial suppression of the active parts, the resultant is "pseudo-dichotomous" (Fig. A. 13). In addition to the facts described above, some inactive marginal cells or cell-group in the apical or subapical region occasionally resumes the meristematic activity resulting in the formation of a new branch or branches.

At first, the gametophyte is prostrate in close contact with the substratum by rhizoids, but in the later stage of its development, partially becomes ascendent off the substratum, often terminates in the group of filamentous gemmae spreading in every direction (Fig. B. 1, 3, Fig. D. 1, 4).

In the older culture, uniseriate filamentous outgrowths come out from the margins of the prostrate part of the thallus. They are often branching in the same way as of the mother filament. Owing to the growth patterns as explained above, whole the structure of the prothallium is very complicated and irregular in outline, giving the impression of ambiguity and difficulty of formulation to the observers (Fig. B. 1-3).

3) Rhizoids: Rhizoids ordinarily grow on the filament cells, on the marginal cells, and most frequently on the cells of ventral side of the

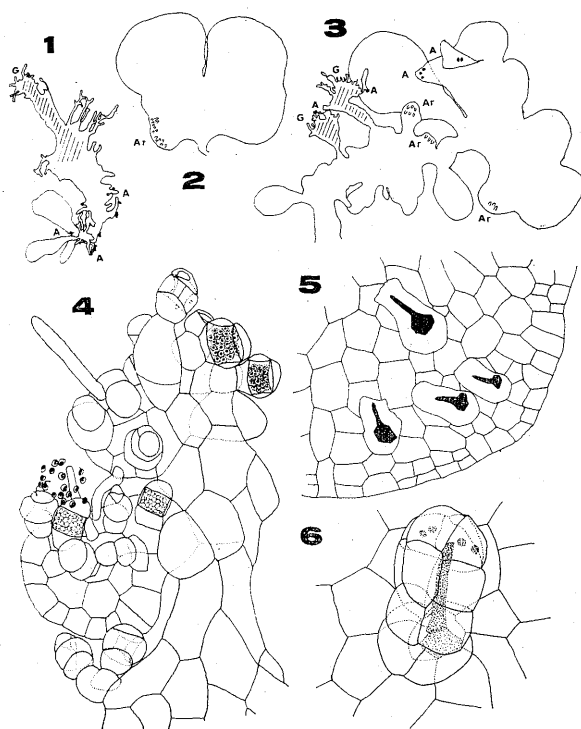


Fig. B. Mature gametophytes with sex organs (1-3. $\times 5$; 4-5. $\times 75$; 6. $\times 200$). 1. 1.5 years, mature thallus with gemmae (G) and antheridia (A). 2-6. 2 years. 2. Thallus with archegonia (Ar). 3. Thallus with gemmae, antheridia and archegonia on marginal cushions. 4. Antheridia on margins of thallus. 5. Cushion with archegonia. 6. Archegonia. (Note: Hatched areas denote manifestly ascending portions of thalli).

prostrate or ascendent thallus. Rhizoids are slightly pigmented with brownish tint. Branching rhizoids are found in a very rare case.

4) Antheridia: Antheridia-formation occurs after a year from the beginning of the culture. It may occur both before and after the gemma-formation. Antheridia-formation begins at the protonema, then proximal margins of the thallus, and finally intramarginal zone on ventral surface of the distal lobe (Fig. B. 1, 3). Antheridia are often produced on the germinating gemmae.

The antheridium is provided with a cap cell, a ring cell, and a barrel-

shaped basal cell (Fig. B. 4). The last mentioned is slightly pigmented, often elongated and nodding (Fig. B. 4). The upper wall of the basal cell is usually flat. In optical section, the numbers of spermatozoid are 24-32 per antheridium. The size of antheridium is $64 \times 80 \mu$ on the average.

5) Archegonia: About a year and half after sowing, archegonia are produced on the cushions of the prothallium. The cushions first mature on the basal margin and later on the apical or subterminal margins of the lobed thallus (Fig. B. 2, 3). The cushion is of one cell in thickness, except for the two-layered cells around the venters of the archegonia (Fig. B. 5). The number of archegonia varies from one to fourteen per cushion (Fig. B. 2, 3). At maturity, archegonia are provided with slightly recurved necks consisting of four to six cell-tiers (Fig. B. 6). The size of archegonium is $67 \times 120 \mu$ on the average.

6) Gemmae: The vegetative reproduction is accomplished by gemma-formation, which appears at the ascendent branches of the thallus after six months from the beginning of the culture. The gemma is a rod-like filament composed mostly of eighteen cells, but sometimes ranging to forty-seven cells (Fig. C. 2, Fig. D. 1, 4, 6). The cell of the gemma is almost filled with chloroplasts and when it is detached from the thallus and is laid down on a suitable medium, it germinates after a short period of rest to form a new prothallium. Gemma first develops only from the marginal cells of the ascendent branches (Fig. B. 1, 3, Fig. D. 1, 4), but later on from the ventral surface of the thallus (Fig. D. 5).

Figure E shows the sequence of gemma-formation. After a small spherical cell is cut off from a marginal cell (P) near the apex, it takes a flask-like shape. Then a terminal cell is cut off at the place of constriction to give rise to an initial cell of gemma (abbreviated as ICG in the text below), leaving the lower cell as a gemmifer (Gf). The former cell rarely repeats to cut off a new terminal cell as in the same manner explained above. The ICG is then divided by several transverse walls to form a gemma (G) (Fig. C. 1, Fig. D. 2, 3). In some instances two ICGs are coupled in series, to make longitudinally arranged gemmae. In general, two to five secondary gemmifers are produced on a primary gemmifer (abbreviated as IGf), the case with 3 Gf being the most usual.

In the same procedure as of the secondary, gemmifers of tertiary and

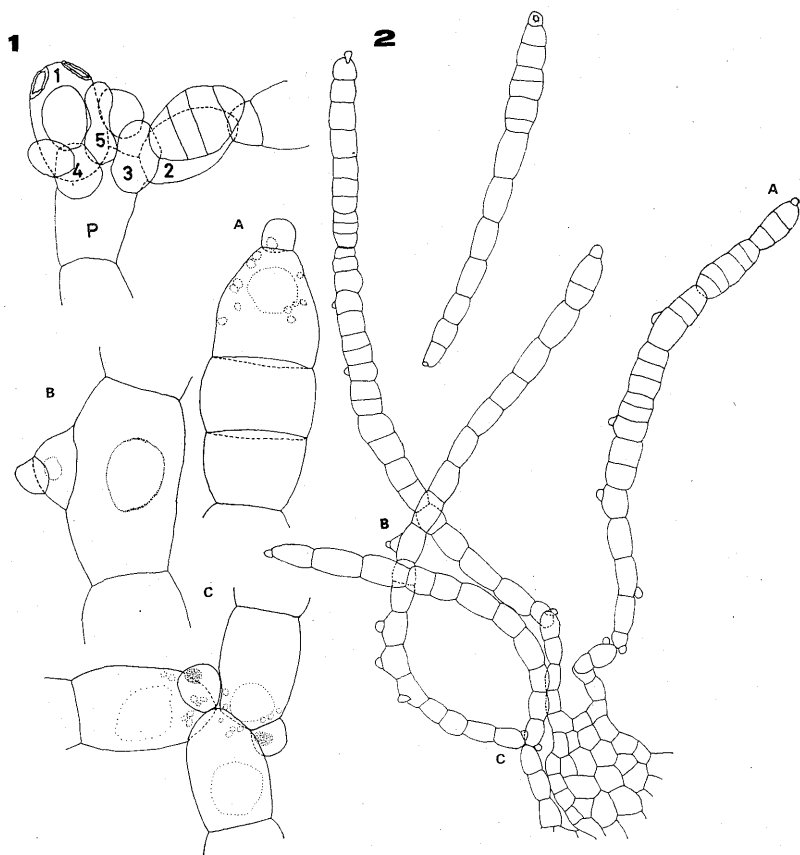


Fig. C. Gemma-formation of 2 years culture (1. $\times 200$; 2. $\times 500$; A-C. $\times 200$). 1. Prothallial cell with 5 gemmifers or gemmifer initials, each with or without gemma. 2. Filaments of gemmae from the apex of thallus, with rhizoidal initials and new intercalary walls. A. Terminal portion. B. Beginning of branching. C. Bases of two gemmae on a gemmifer. (A-C on the left correspond the same letters on the right).

quaternary generations are seen to be produced successively on a prothallium. Generation of Gf is symbolized by the letter I, II, . . . and so on. Every generation may have their fraters. For example, in IGf they are 1-5 in number (abbreviated as IGf_{1-5}). The results of the observation is formulated as follows, IGf_{1-5} , $IIIGf_{1-3}$, $IIIGf_{1-3}$, and $IVGf_{1-3}$. In the similar way the generation and fraternity of gemmae, as far as the author has

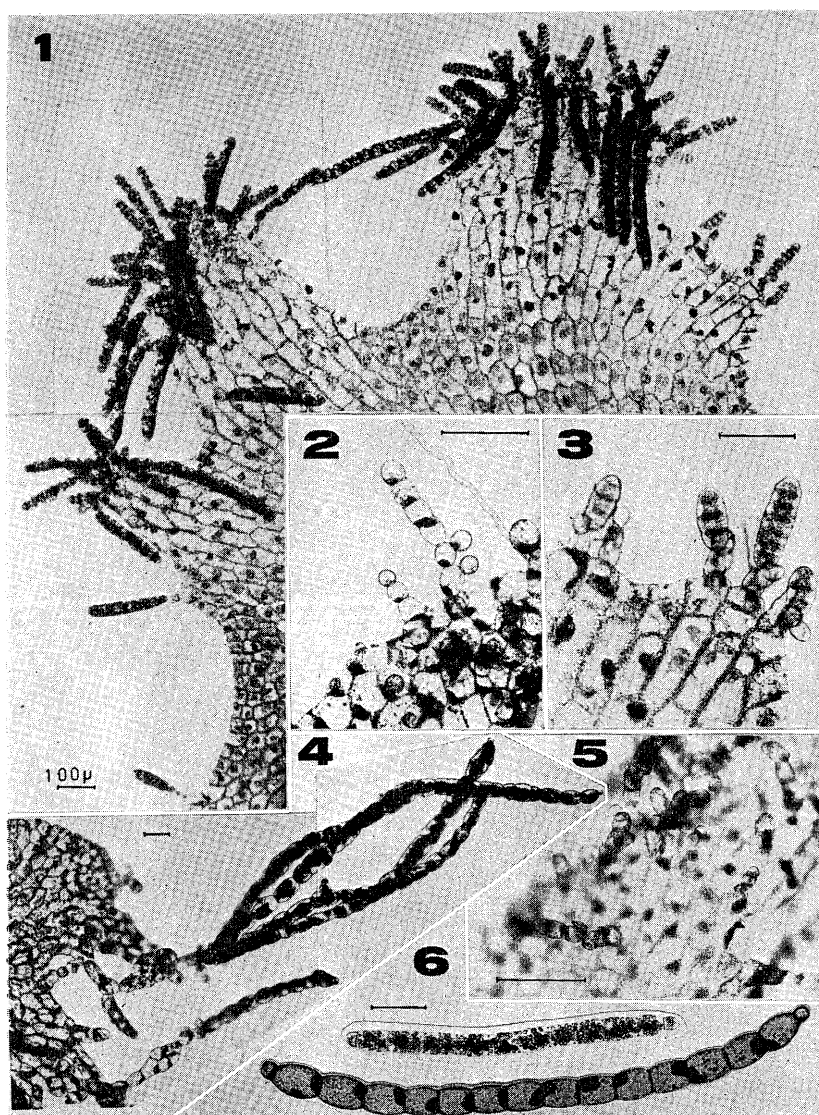


Fig. D. Gemma-formation of 2 years culture. 1. Early stage, gemmae on the marginal portion. 2. Earlier development of gemmae and gemmifers. 3. Magnified photo of the right end of D-1. 4. Later stage. 5. Gemma-formation on ventral surface. 6. Mature and juvenile gemmae with two rhizoidal initials on their both ends. (Horizontal line in each photo denotes 100μ).

observed, may be formulated as follows: IG_{1-5} , IIG_1 .

Shortly prior to the maturation, the cells at both ends of the gemma cut off rhizoidal initials. After a short period of dormant stage, the rhizoidal initials begin to elongate to form the first rhizoids. A comparatively small number of the intervening cells also cut off rhizoidal initials, mostly 1-6 in number (Fig. C. 2, Fig. D. 6). Two rhizoidal initials are very rarely observed to come out from a single cell. When the rhizoidal cell initials are completed in formation, they are slightly pigmented with brownish tint, with or without observable chloroplasts. In the meantime, the abscission follows at the place of attachment of the gemmae. The primary gemmifer (IGf), however, persists to keep touch with the thallus. A small circular scar is formed by the abscission, on both of the gemmifer and the gemma (Fig. C. 1). The body cells of the gemma, excepting the terminal ones, may produce laterally simple uniseriate branches. In this case the gemma is detached from the gemmifer with a branched shape. No abscission takes place at the point of ramification. The longitudinal wall formation is also rarely observed in the body cell. In the old thallus, gemmifer turns into a small plate-like gemma with the transverse and longitudinal cell wall formation (Fig. D. 4). During the gemma-formation, the marginal cell P defined as above begins to elongate and divide into a short filament, consisting of several cells.

Discussion The gametophyte of *Vittaria Fudzinoi* Makino is ribbon-like, and agrees with those of the previously reported vittarioid ferns. Nayar and Kaur¹⁸⁾ termed this gametophyte development "Kaulinia type", which they described to be most frequently found in Vittariaceae, Hymenophyl-

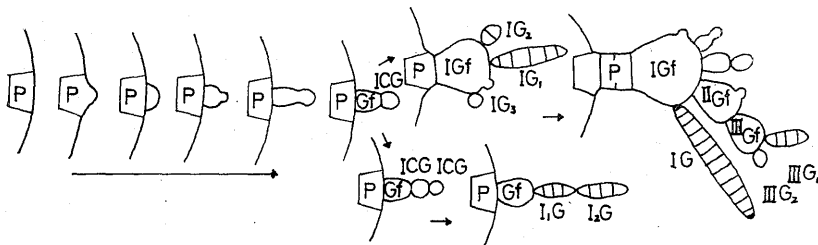


Fig. E. The schematic representation showing the process of gemma-formation. Refer to the text.

laceae and Polypodiaceae. The pattern of germination is "Tangential" type as proposed by Momose¹¹⁾, and is designated as "Vittaria-type" by Nayar and Kaur¹³⁾. The occurrence of the second and the third filaments originated from the basal cell is somewhat similar to those of Hymenophyllaceae and Grammitidaceae. This kind of development seems to be a unique phase in the epiphytic group of ferns.

The prothallium is of one cell in thickness except for the portions where archegonia are produced. The external structures and shapes of both sex organs are very similar to those of the leptosporangiate ferns with cordate prothallia. The antheridia are produced independently of gemma-formation against the Farrar's view⁷⁾ that the gemma-formation seems to induce antheridia. These are produced at first on the marginal portion, then scattered on the ventral surface, and finally on the gemma. The gemma-formation is of regular sequence, but the position of the occurrence is variable.

In this respect further studies are still needed to clarify the classification and phylogeny of vittarioid ferns and their allies.

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Literature

- 1) Atkinson, L.R. 1973. Bot. J. Linn. Soc. 67 (supp. 1): 74-90.
- 2) Bower, F.O. 1926-1928. The ferns Vol. 2-3.
- 3) Britton, E.C. and A. Taylor 1902. Mem. Torrey Bot. Club, 8: 185-220.
- 4) Copeland, E.B. 1947. Genera Filicum.
- 5) — 1951. Phil. Journ. Sci. 80(2): 97.
- 6) Farrar, D.R. 1967. Science 155: 1266-1267.
- 7) — 1974. Amer. J. Bot. 61(2): 146-155.
- 8) Goebel, K. 1877. Bot. Ztg. 35: 671-678, 681-694, 697-711.
- 9) — 1888. Ann. Jard. Bot. Buitenzorg 7: 74-119.
- 10) — 1896. Flora 82: 67-75.
- 11) Momose, S. 1942. Bot. Mag. Tokyo 18(2): 49-65.
- 12) — 1967. Prothallia of the Japanese ferns (Filicales).
- 13) Nayar, B.K. and S. Kaur 1969. Phytomorphology 16: 179-188.
- 14) — 1971. Bot. Rev. 37: 295-396.
- 15) Stokey, E.G. and R. Atkinson 1958. Phytomorphology 8: 391-403.
- 16)

— 1964. Ibid. 14: 51-70. 17) Wagner, W.H.Jr. and A.J. Sharp 1963. Science 142: 1482-1484. 18) Yoroi, R. 1972. The Science Report of the Tokyo Kyoiku Daigaku Sec. B. 15(225): 81-110.

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日本と中国に固有の種であるナカミシランの配偶体を孢子（面河溪産）からの培養実験によって観察した。配偶体は不規則に分岐したリボン状の葉状体で、無性芽を生じることが特徴的である。孢子の発芽様式は百瀬博士の接線発芽を示す。また Nayar & Kaur によれば *Vittaria*-type を示す。前葉体の発達型については N. & K. の *Kaulinia*-type を示すが、コケシノブ科やヒメウラボシ科と似かよった初期前葉体形成の様式も観察した。配偶体の細胞は造卵器が形成される位置以外は一層である。造卵器・造精器の構造と外部形態は他の多くの薄のウシダ類と似かよっている。造精器の発生する位置は成熟度によって移動するが、これは無性芽の発生と関係がないことが判明した。無性芽は葉状体の縁や腹面に形成され、この種での発生様式は安定していると考えられるので、その過程を詳細に報告した。

□奥山春季：採集検索 日本植物ハンドブック pp. 783 八坂書房、東京 (1974)。本書は 1953 年に出た植物採集ハンドブックの増補版の形をとるが、内容的には全く一新されている。即ち独立した 3 部から成り、夫々が意味を持っているといつてよい。第一部は地域別の植物で日本の各地方（小笠原と沖縄を除く）の主な採集地 188 についてそこでの注目すべき種名を網羅したが、特にそこをタイプロカリティとするものは変種品種までゴチックで明示し、さらに何か特徴的な植物の図を添え、また文献を挙げた。よくみると注意すべきものには文献や二三の注目すべき点も添記されていて、各地の植物相の概観を知り、特徴を掴むのにまことに打ってつけである。これは多年科学博物館にあって全国の同好家と接しておられた氏を以てはじめてなし得た処であると思う。第二部は近似植物の検索で同属或は近似属の類似種間の識別を記したもので要を得ている。第三部は分類植物名鑑で、シダ類以上の日本に生ずる種名を、できる限り簡略に列記したもので、学名と和名とだけであるが必要に応じて変種、品種、分布、異名も附記されている。栽培種も帰化品も入っていて現在日本に見出される植物名を知るのに最もよい。抄録者のみるところではここが著者が最も力を入れたところであり、属の範囲は中庸で中々味がある。たゞ全体が A B C の順であるのが惜しいところである。科学博物館を停年退職された記念としてまことにふさわしい出版物で、深く敬意を表するものである。

(前川文夫)